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## Uncrossed cortico-muscular projections in humans are abundant to facial muscles of the upper and lower face, but may differ between sexes

**Abstract** It is a popular concept in clinical neurology that muscles of the lower face receive predominantly crossed cortico-bulbar motor input, whereas muscles of the upper face receive additional ipsilateral, uncrossed input. To test this notion, we used focal transcranial magnetic brain stimulation to

quantify crossed and uncrossed cortico-muscular projections to 6 different facial muscles (right and left Mm. frontalis, nasalis, and orbicularis oris) in 36 healthy right-handed volunteers (15 men, 21 women, mean age 25 years). Uncrossed input was present in 78% to 92% of the 6 examined muscles. The mean uncrossed:crossed response amplitude ratios were 0.74/0.65 in right/left frontalis, 0.73/0.59 in nasalis, and 0.54/0.71 in orbicularis oris; ANOVA  $p > 0.05$ ). Judged by the sizes of motor evoked potentials, the cortical representation of the 3 muscles was similar. The amount of uncrossed projections was different between men and women, since men had stronger left-to-left pro-

jections and women stronger right-to-right projections. We conclude that the amount of uncrossed pyramidal projections is not different for muscles of the upper from those of the lower face. The clinical observation that frontal muscles are often spared in central facial palsies must, therefore, be explained differently. Moreover, gender specific lateralization phenomena may not only be present for higher level behavioural functions, but may also affect simple systems on a lower level of motor hierarchy.

**Key words** facial nerve · facial emotions · transcranial magnetic stimulation · gender differences

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### Introduction

Peripheral and central facial palsies are clinically distinguished by examination of the degree of muscle weakness of the upper face. After peripheral facial nerve lesions, the severity of muscle weakness of the forehead equals that of the lower face, whereas with brain lesions in one hemisphere such as in stroke, the frontal weakness is typically much less pronounced than that of the lower face. This clinical observation has been attributed to differences of central motor projections to the upper and lower face. According to a common concept, muscles of the lower face receive mainly crossed projections from the opposite hemisphere, while muscles of the upper face (e.g., the frontalis muscle) receive significant

additional uncrossed projections from the ipsilateral hemisphere, which are assumed to be spared by unilateral hemispheric lesions [1, 3, 30].

A number of observations challenge this traditional concept of central motor pathways to facial muscles. First, clinical experience suggests that the presence of severe frontal weakness is not always indicative of the peripheral origin of a facial palsy, but may be observed in central facial palsies as well. Thus there may be interindividual differences with respect to the strength of the presumed uncrossed projections. Second, anatomical tracing studies in patients with unilateral hemispheric lesions and microstimulation studies in primates suggest that facial nuclei containing motor neurons to upper and lower facial muscles receive abundant projections from both, contra- and ipsilateral

hemispheres [21, 22, 24]. Third, transcranial magnetic cortex stimulation (TMS) often yields ipsilateral motor evoked potentials (MEPs) in muscles of the lower face, as observed by us [15, 36, 37] and by others [27, 28, 43]. In a TMS study, Cruccu et al. confirmed uncrossed projections to upper facial muscles but not to lower ones, but their lower facial responses analysis was possibly biased by recording problems [8]. The present study was undertaken to reinvestigate the presence and amount of uncrossed cortico-facial projections in healthy humans by use of focal transcranial magnetic stimulation.

## Subjects and methods

Thirty-six healthy subjects including two of the authors (15 men, 21 women; mean age 25 years, range 19–57) gave their informed consent to participate in the study, which was approved by the local ethics committee. Right-handedness was confirmed in all of them using the Edinburgh handedness inventory [32].

### Recordings

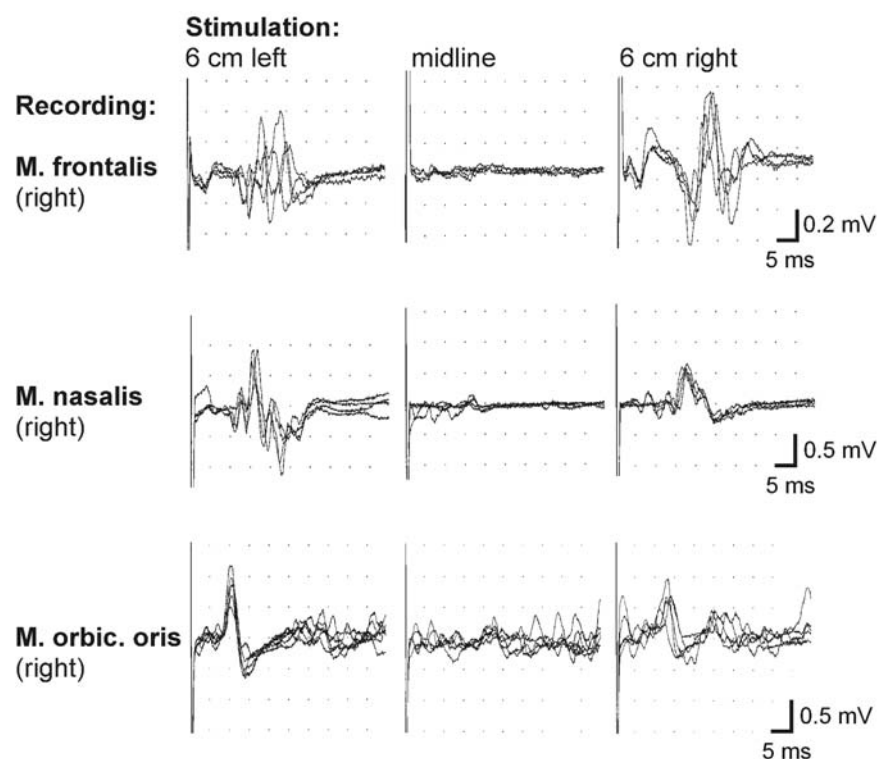
Recordings were made from m. frontalis and m. orbicularis oris ( $n = 11$  men and 13 women), and from m. nasalis ( $n = 15$  men and 21 women) on both sides. Surface electrodes were used, with the reference electrode taped over the presumed motor point and the indifferent electrode 1 cm laterally. Compound muscle action potentials (CMAPs) from such close electrode montage may be polyphasic and begin with either positive or negative deflections (Fig. 1). Despite this disadvantage, this montage was chosen to prevent interference by vol-

ume conducted activity from other facial muscles. Pilot experiments using these montages along with intramuscular concentric needle recordings demonstrated equivalent results with either method. To exclude recording of volume conduction from the contralateral side of the face, a supramaximal stimulation of the facial nerve in the stylomastoid fossa was performed at the beginning of each experiment. If this peripheral stimulation elicited contralateral muscle responses (by volume conduction), the recording electrodes were replaced until such responses were absent. For the recordings from m. frontalis, a stand alone device for suppression of the stimulus artifact was used [31]. Bandpass filtering was 2 Hz – 5 kHz. A Viking III EMG apparatus (Nicolet, Madison, USA) was used for the recordings.

### Stimulations

Stimulations were performed using a Magstim 200 stimulator (The Magstim Company Ltd., Spring Gardens, Whitland, U.K.), equipped with a standard focal figure-of-eight stimulation coil. Stimuli were delivered by placing the coil with its handle pointing backwards over three different locations along the interauricular line, namely over the vertex, and 6 cm to the left and to the right. The antero-posterior coil orientation was chosen to reduce the risk of co-stimulation of the contralateral hemisphere. While this orientation may not always be optimal to elicit the largest possible motor evoked potential (MEP), it is nonetheless suited to yield near maximal MEPs [11]. Small adjustments of the coil position were done to optimize the muscle responses. Reliable threshold measurements were not possible in the 3 investigated facial muscles, because facilitation by slight voluntary precontraction was almost always needed to obtain responses, and contractions of facial muscles are difficult to quantify. Therefore, to standardize stimulation, the stimulation intensity was stepwise increased at the beginning of each experiment, just until the size of the MEPs obtained from the contralateral muscles became saturated (i. e., did not further increase with increasing stimulation intensities). The same stimulation intensity was used for the 6 investigated muscles of

**Fig. 1** Responses from three different right-sided muscles of the upper and lower face, after transcranial magnetic stimulation over the left and right hemisphere, and over the vertex. Stimulation over the vertex (midline) does not evoke responses in either muscle while stimulation over both hemispheres evokes well reproducible responses, with nearly symmetrical latencies and amplitudes



each subject, and it was kept constant during all stimulations. Pilot experiments had shown that with these stimulation settings, no blink reflex response was obtained. During the stimulations, the subjects performed a slight symmetrical voluntary contraction of the target muscles to facilitate the response [19, 35]. To compensate for the inherent variability of motor evoked potentials [12], 6 to 8 stimuli were given for each muscle and each coil position to collect a representative sample of responses.

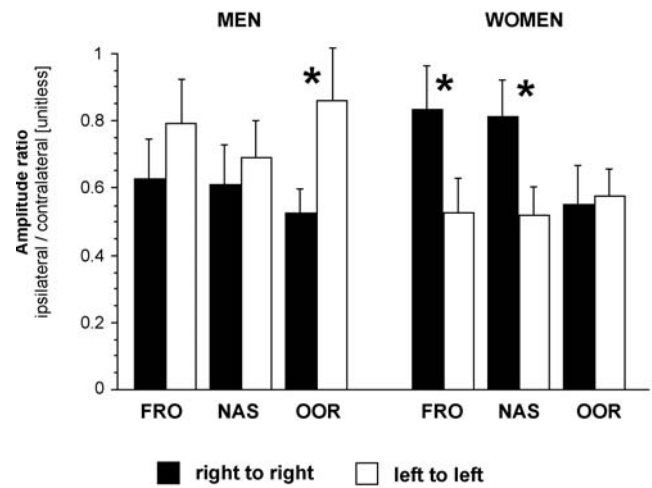
#### Data analysis

Onset latencies of the muscle responses were measured to the first positive or negative deflection from the base line. Amplitudes were measured peak to peak. For further analysis, the largest amplitude and the shortest latency of the 6–8 recorded responses were chosen. If there was no reproducible response, the amplitude was considered to be 0 mV. The amount of uncrossed projection was calculated for each muscle as the amplitude ratio of the ipsilateral and the contralateral response ( $MEP_{ipsilat.}/MEP_{contralat.}$ ). To quantify the amount of the cortical projections to the facial nucleus, the amplitude ratio of cortical and peripheral responses was calculated ( $MEP_{contralat.}/CMAP_{periph.}$ ). For comparison of group means, Student's *t* test for paired or unpaired comparisons was used. For comparison of the  $MEP_{ipsilat.}/MEP_{contralat.}$  ratio between the 3 pairs of muscles, a repeated measures analysis of variance (ANOVA) was done. The level of significance was set to  $p = 0.05$ .

## Results

In all muscles from all subjects, responses could be evoked by TMS of the contralateral hemisphere. These muscle responses had the typical characteristics of motor evoked potentials, i. e., they were facilitated by ongoing slight voluntary contractions, and they varied in shape and size from one stimulus to the next (Fig. 1). Responses in m. frontalis were easily obtained after elimination of the stimulus artifact by a previously described device [31]. The frontalis responses had similar latencies to those from nasalis and orbicularis oris. Their amplitudes were somewhat smaller (Table 1).

Magnetic stimulation of the ipsilateral hemisphere evoked responses in the majority of muscles and subjects (Table 1, Fig. 1). The amplitude ratio of  $MEP_{contralat.}/CMAP_{periph.}$  was greater in the frontalis muscle than in the nasalis and orbicularis oris muscles



**Fig. 2** Size of the muscle responses to uni-hemispheric magnetic stimulation in ipsilateral muscles. Given is the ratio between amplitudes in ipsilateral vs. contralateral muscles, separately for men and women. Bars indicate standard errors of the mean. \* Indicates  $p < 0.05$ , in paired *t*-tests; FRO frontalis muscle; NAS nasalis muscle; OOR orbicularis oris muscle

(Table 1), thus indicating that the crossed cortico-bulbar projections subserving the frontalis muscles were at least as strong as the ones to the lower face muscles.

There was no significant difference between the muscles of the upper and those of the lower face with respect to the percentage of muscles in which ipsilateral TMS evoked responses (range between 78% and 92%; ANOVA  $p > 0.05$ ; Table 1). The amplitudes and the amplitude ratio of  $MEP_{ipsilat.}/MEP_{contralat.}$  were not different between the 6 examined muscles, if data from men and women were pooled (ANOVA  $p > 0.05$ ; Table 1). The onset latencies were virtually symmetrical for crossed and uncrossed projections, and suggested oligosynaptic pathways. Thus, muscle responses on the side of the stimulated brain hemisphere were not mediated through transcallosal connections or through polysynaptic interconnections crossing back over the midline at the level of the brain stem.

The proportion of uncrossed projections was differ-

**Table 1** Muscle responses to brain stimulation in 6 muscles (= MEPs). Given are means  $\pm$  1 standard deviation. Data for men and women are pooled

	side	Number of responses uncrossed	Amplitudes [mV]*		$MEP_{ipsilat.}/MEP_{contralat.}$ ** [amplitude ratio]	$MEP_{contralat.}/CMAP_{periph.}$	Onset latency [ms]	
			crossed	uncrossed			crossed	uncrossed
Frontalis <i>n</i> = 24 muscle pairs	r	20 of 24 (= 83%)	0.53 $\pm$ 0.055	0.35 $\pm$ 0.042	0.74 $\pm$ 0.426	0.56 $\pm$ 0.222	10.2 $\pm$ 1.30	11.0 $\pm$ 1.58
	l	20 of 24 (= 83%)	0.55 $\pm$ 0.056	0.35 $\pm$ 0.052	0.65 $\pm$ 0.414	0.67 $\pm$ 0.252	11.7 $\pm$ 1.30	11.5 $\pm$ 2.17
Nasalis <i>n</i> = 36 muscle pairs	r	31 of 36 (= 86%)	1.03 $\pm$ 0.095	0.65 $\pm$ 0.070	0.73 $\pm$ 0.487	0.37 $\pm$ 0.189	10.3 $\pm$ 1.66	10.4 $\pm$ 1.23
	l	28 of 36 (= 78%)	0.97 $\pm$ 0.096	0.55 $\pm$ 0.083	0.59 $\pm$ 0.406	0.33 $\pm$ 0.174	11.5 $\pm$ 2.08	11.2 $\pm$ 2.45
Orbicularis oris <i>n</i> = 24 muscle pairs	r	19 of 24 (= 79%)	1.09 $\pm$ 0.133	0.50 $\pm$ 0.072	0.54 $\pm$ 0.341	0.32 $\pm$ 0.187	9.4 $\pm$ 1.43	10.6 $\pm$ 1.29
	l	22 of 24 (= 92%)	1.20 $\pm$ 0.133	0.77 $\pm$ 0.096	0.71 $\pm$ 0.434	0.33 $\pm$ 0.200	10.5 $\pm$ 1.95	10.6 $\pm$ 2.55

\* If no response was evoked, amplitude was assumed to be 0 mV; \*\* Mean of individual ratios

ent between women and men. In women, uncrossed right-to-right projections were stronger than left-to-left projections, this difference reaching statistical significance in frontalis and nasalis (Fig. 2). In men, left-to-left projections were more powerful than right-to-right projections, reaching statistical significance in orbicularis oris (Fig. 2). Thus, in men more uncrossed projections originated from the left (dominant) hemisphere, while in women, more uncrossed projections originated from the right (non-dominant) hemisphere ( $p < 0.05$ ).

## Discussion

Our study demonstrates that in healthy humans, there are abundant uncrossed cortico-muscular pathways to muscles of the upper and lower face. Facial muscle responses were found ipsilaterally to the stimulated hemisphere in most subjects (Table 1). Their size averaged between 46% and 67% of the responses to contralateral stimulation, and there was no difference between the three examined muscles in this respect. These findings challenge the traditional textbook concept explaining the difference between central and peripheral facial palsies [1, 3, 30]. Hence, sparing of muscles of the upper face in central facial palsies may have an explanation different from the presence of undisturbed uncrossed cortico-muscular projections.

It is unlikely that our findings could be explained by nonfocal stimulation or recording. For magnetic brain stimulation, a focal figure-of-eight coil was used, which was shown to activate cortical motor neurons selectively on one hemisphere. Using similar stimulation parameters for small hand muscles of healthy subjects, only responses in contralateral muscles have been obtained after hemispheric stimulation by a figure-of-eight coil [34]. In our subjects, stimulation over the vertex never evoked a response in any of the six muscles (Fig. 1), sufficiently demonstrating focal stimulation. Unilateral stimulation of the supraorbital nerve may elicit bilateral, reflexive muscle responses in orbicularis oculi, with a latency of about 10 ms [23]. It is, however, unlikely that we recorded such blink reflex R2 responses, since, as pointed out above, stimulation over the vertex did not elicit responses. Moreover, blink reflex R2 responses to supraorbital nerve stimulation are predominantly observed in orbicularis oculi muscle, but not in orbicularis oris. Hence, our finding of ipsilateral responses in orbicularis oris cannot be explained by a blink reflex mechanism. Finally, volume conducted activity from the contralateral side of the face was ruled out by careful adjustments of the recording electrode position during peripheral stimulation of the contralateral facial nerve in each of the subjects.

Uncrossed cortico-muscular projections have been described using (intra-)cortical stimulation methods to

a variety of limb muscles in animals [14, 21] and in man [33]. In general, uncrossed projections were found to be more abundant in proximal than in distal muscle groups, and had greater latencies and higher stimulation thresholds than crossed projections [16], suggesting polysynaptic (extrapyramidal) pathways. The projections described here are different, since they were elicited using symmetrical stimulation strength, and the latencies were symmetrical as well (Table 1). Magnetic brain stimulation predominantly examines the fast conducting monosynaptic “pyramidal” motor pathway [20]; and the characteristics of the uncrossed projections demonstrated here are those of this pathway. Thus, our responses were not conducted via the corpus callosum, and an oligosynaptic circuit crossing sides back again at the level of the brain stem seems unlikely. Our findings corroborate the results of Cruccu et al. [8], who “often” found ipsilateral responses in lower facial muscles of their 10 subjects, but rejected these responses as proof of uncrossed projections because they could not rule out the possibility of volume conduction from the contralateral side of the face. Such volume conduction was excluded by the present experimental set up.

As stated above, the relative preservation of frontal muscle force in central facial palsies is probably not explained by differential importance of uncrossed projections. In fact, in one of the most influential studies on this subject, Kuypers [24] found abundant uncrossed input to the facial subnuclei subserving the lower facial muscles using neuroanatomical tracing techniques in four unilaterally damaged human brains, a result well in line with our present data. Similar findings have been made in monkeys using anterograde labelling of cortico-nuclear neurons by horseradish peroxidase [22] and by cortical microstimulation techniques [21]. Alternative explanations for the clinical observation are beyond the scope of this paper, because we did not examine patients. It has been speculated that the cortical representation of the frontal muscles is smaller than that of lower facial muscles, and hence their activation depends more on alternative (subcortical and involuntary) circuits [22]. Therefore, in hemispheric lesions they might not be affected to the same extent as muscles of the lower face. Our data allow an estimate of the strength of corticobulbar projection, by analysis of the ratio of MEP size (which relates to the amount of cortico-muscular outflow) and CMAP<sub>periph.</sub> size (which relates to the amount of motor units and hence bulbar outflow). The MEP<sub>contralat.</sub>/CMAP<sub>periph.</sub> ratio was greater in frontalis than in nasalis and orbicularis oris, indicating that the strength of corticobulbar projections in frontalis was not less important than that of the nasalis and orbicularis oris muscles (Table 1). Moreover, the stimulation intensities necessary to obtain MEPs were not different for the three muscle pairs. Thus, our data do not support



the suggestion that smaller cortical representation of upper facial muscles causes sparing of the upper face in central facial palsies. Nonetheless, our data do not exclude the possibility that there is a greater amount of subcortico-bulbar input to the muscles of the upper face, which may eventually explain the clinical observation.

A large body of research on the hemispheric lateralization of emotions assumes that facial muscles of the lower face receive their input predominantly from one hemisphere. Under this assumption, one hemisphere is thought to control the facial expression of the contralateral side of the face [2, 4, 7, 29]. Observation of side differences in facial expressions might thus allow drawing conclusions on differential hemispheric involvement in emotional processes. This assumption was previously challenged on grounds of neuropsychological data, as critically discussed by Thompson [42], and Hager & Ekman [17]. Also the present findings do not support the validity of this assumption, since they demonstrate an important input from both hemispheres upon either side of the lower face. Side to side differences of facial expressions implying emotions in healthy subjects should therefore be interpreted carefully with regard to hemispheric lateralization; and such findings

need to be compared with those from patients suffering from unihemispheric brain lesions [38].

As an unexpected result we found a difference between men and women with respect to the importance of uncrossed projections to facial muscles. Left-to-left projections were more abundant in men, and right-to-right projections were stronger in women (Fig. 2). Gender differences have been postulated in higher order cerebral functions such as production and appreciation of emotions [6] or cognitive processing of spatial and logical tasks [26, 44]. The right hemisphere is more involved in the perception and in the facial expression of emotions [13, 18, 39, 41], and a number of studies indicate that women are more facially reactive to emotional stimuli than men [5, 9, 10, 40]. Thus, women show greater right hemispheric involvement than men in facial responses to emotional stimuli [25]. Even though activation of facial miming is only one aspect of emotional expressions, the present results are in good agreement with these findings, since they suggest a greater importance of right-to-right connections in women than in men (Fig. 2). Our results indicate that such gender differences of facial expressions may relate to differences of the cortico-nuclear projections rather than to differences of higher order emotional processing.

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